and is characterized by an instability leading to the release of short, abortive RNA products.
It was proposed many years ago that the release of abortive products results from a growing ''stress'' in the system. Recent crystal structures have suggested that ''scrunching'' of the template (single) strand connecting the initiation site to the duplex promoter region contributes substantially to this stress.
In order to probe the role of this connecting single strand, we have created various constructs in which regions of this single stranded DNA have been nicked, deleted (gapped) or overlapped and then have asked how this effects both 1) the initial positioning of the template DNA and 2) the stability of constructs stepping away from the promoter (abortive cycling). These results provide a direct test of the scrunching model for stress during the initially transcribing phase of transcription.

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Prevention Of Backtracking Alleviates Nucleosomal Barrier To Transcription
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Nucleosomes are fundamental repeating subunits of eukaryotic chromatin which help to package DNA tightly into the cell nucleus. During transcription, RNA polymerase (RNAP) must be able to transcribe the DNA associated with nucleosomes, yet nucleosomes are known to be major barriers to transcription. Here we use optical trapping techniques in combination with biochemical methods to study the mechanisms by which E.coli RNAP transcribes through nucleosomes. Although E. coli RNAP never encounters chromatin in vivo, its core enzyme is evolutionarily conserved and shares homology in sequence, structure and function with eukaryotic Pol II, suggesting that E. coli RNAP may be a simple system to study transcription through nucleosomes. We have constructed DNA templates, each containing a T7A1 promoter followed by a well-positioned mononucleosome. We use optical trapping to detect with high precision the position and structure of proteins before and after transcription. Our results show that RNA polymerase may backtrack when it encounters a nucleosome. Prevention of backtracking alleviates the nucleosomal barrier, promoting more efficient transcription through nucleosomes.

286-Pos Board B165
Monte Carlo Simulation of Transcriptional Control in 6kbp DNA
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Eukaryotic genes are regulated by gene-control regions such as enhancers and insulators. Enhancers are regulatory DNA sequences that can activate transcription of genes located at large distance. Insulators are regulatory DNA sequences that can inhibit transcription when they are located between an enhancer and a promoter. Recently, it has been reported that the enhancer is in physical proximity to the active genes in vivo with the intervening DNA looping out [1]. It has also been indicated that insulators may modulate the enhancer-promoter interactions by interacting with each other and facilitating the formation of chromatin or the DNA loop domains [2, 3]. In this way, importance of the DNA or chromatin conformation at transcriptional control has begun to be recognized. However, by only using molecular biological techniques, we can hardly see the relationship between the atomic level information of DNA and the transcriptional control. Thus, in order to investigate effects of atomistic and thermodynamic properties of DNA on the enhancer-blocking activity, we performed Monte Carlo simulation with the wormlike chain model of DNA. Results are consistent with the experimental data of Ameres et al. [3], showing that the amount of gene expression is proportional to the relative proximity probability between an enhancer and a promoter. Furthermore, our results suggest that the proximity probability between these two elements is reduced because the stretching and bending energies increase when the formation of DNA loop domains modulates the enhancer-promoter interactions.
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[3] S.L. Ameres et al.,EMBO J. 24, 358 (2005).

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Investigating The Structural Dynamics Of The Lict Transcriptional Anti-terminator And Its RNA Target By Single Molecule FRET
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LicT belongs to a family of bacterial transcriptional anti-terminators that regulates the expression of sugar metabolizing operons through a riboswitch mechanism. When activated, these proteins bind to a short RNA hairpin in the 5′UTR of their target mRNAs and thereby prevent the formation of an overlapping transcriptional terminator. Previous structural studies of conformational changes have revealed the structural basis of RNA recognition by the N-terminal RNA-binding domain and the phosphorylatable regulation domain upon activation (1). The current model of the LicT regulation mechanism posits that the inactive protein oscillates between several open dimeric conformations. Activation via phosphorylation (or by constitutive mutations) locks the LicT dimer in a unique closed conformation which can efficiently bind and stabilize the anti-terminator RNA hairpin. We investigated the conformational dynamics of LicT and its target RNA using single molecule Forster Resonance Energy Transfer (smFRET) with Alternating Laser EXcitation (ALEX). By this technique, we obtain quantitative information on the magnitude of the structural changes undergone by the LicT protein domains and the RNA molecule in the active/inactive and free/bound states. We observe different mutations to quantify how they affect the thermodynamic equilibrium and dynamics.
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Characterization of The Open Complex of Yeast Mitochondrial RNA Polymerase
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The mitochondrial RNA polymerase (mtRNAP) of Saccharomyces cerevisiae, consisting of a complex of Rpo41 and Mtf1, is homologous to the phage single polypeptide T7/T3/SP6 RNA polymerase and mammalian mitochondrial RNA polymerases. The yeast mtRNAP recognizes a conserved nonanucleotide sequence to initiate specific transcription. However, there is little clue about how mtRNAP recognizes the promoter, melts the double-stranded DNA and forms a transcription-competent open complex, which are steps essential to initiating RNA synthesis. In this work, we have defined the region of the nonanucleotide melted by the mtRNAP using 2-aminopurine fluorescence changes that are sensitive to changes in DNA base stacking interactions. We have also characterized DNA bending of the promoter induced by mtRNAP by fluorescence resonance energy transfer (FRET) measurements. We show that DNA melting requires the simultaneous presence of Rpo41 and Mtf1, whereas DNA bending can be induced by Rpo41 itself and regulated by MtF1. By monitoring DNA melting and bending under various conditions, we are able to dissect roles of Rpo41 and MtF1 in DNA recognition and forming the open complex. These results shed new insights into mechanisms of transcription initiation of mitochondrial RNA polymerases.

289-Pos Board B168
Characterizing the Effects of Highly Bent DNA on Transcription
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It is well-established that many transcriptional repressors such as the loop-forming lactose or galactose repressors tightly bend DNA. However, it remains unclear whether this bending directly affects the activity of RNA polymerase. Characterizing the effects of repressor-bound, highly stressed DNA on transcription is complicated by the inability to decouple the mechanical state of the DNA template from the kinetics of repressor binding, which itself will be influenced by the energy required for the repressor to strain the DNA. To our knowledge there currently exists no established assay capable of quantifying transcription by RNA polymerase from highly bent DNA templates which mimic the bending induced by loop-forming repressors in the absence of other DNA-binding proteins. We have developed a fluorescence-based *in vitro* assay capable of addressing this experimental limitation by exploiting the ability of DNA minicircles to impose both varying degrees of bending and twist. We are now applying this assay to characterize the rates of transcription by T7 RNA polymerase from highly stressed templates. We hypothesize that the activity of T7 RNA polymerase will be reduced on templates that are bent with degrees of curvature comparable to the loops generated by loop-forming repressor proteins. Contrary to our initial hypothesis, preliminary data suggests that at least one such highly bent DNA template is in fact readily transcribed by T7 RNA polymerase. However, the degree to which the minicircle templates are twisted beyond the torsionally relaxed helical structure may play a greater-than-expected role on the activity of RNA polymerase, and must therefore be addressed. Conclusions resulting from these measurements will shed light on the mechanism by which loop-forming repressors affect transcription, and could reveal a more significant role for the mechanical state of the DNA than was previously thought.